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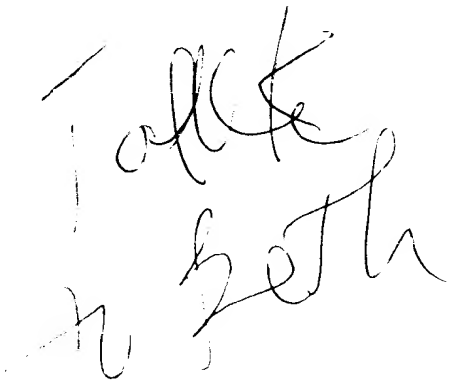
Message



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SENT BY FACSIMILE

Québec, August 28, 2003

Ms. Georgia L. Helmer
Art Unit 1638
USPTO
Commissioner of Patents
B.O. 1450
Alexandria, Virginia 2231301450

Dear Ms. Helmer:

RE: US Patent Application No. 09/678,303 – October 3, 2000
PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES
Inventors: Louis-Philippe Vézina et al.
Our Ref.: 14149-4US

This follows our telephone conversation of yesterday (August 27, 2003) regarding the above-mentioned patent application in the USPTO. As proposed, I send you a draft, identified as such, of an amendment of page 5 of the disclosure and of the claims of US patent application number 09/678,303 in order to eliminate the enablement aspect due to the citation of the plasmid pGPLAS3.2 on the third line of the fist paragraph of page 5. This citation has been replaced by identification of SEQ ID NO:1 and "deletion fragment" was replaced by SEQ ID NO:2 and SEQ ID NO:3. It is believed that this would clarify this part of the protocol to allow someone skilled in the art to perform the claimed invention. The matter would now be restricted to SEQ ID NO:1, 2 and 3.

The claims have been accordingly amended. The Applicant wishes to limit the expression of DNA of interest in leaves of transgenic plants, and a claim 2 is deleted from the application and the claim 5 is limited to plants from the group of alfalfa.

The review of this **draft** amendment I respectfully propose to have a telephone discussion at your time and convenience.

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Sincerely yours,

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PMMG/np
Enclosure

HindIII and EcoRI sites of the pUC19 polycloning site. The resulting plasmid was named pBI201 and was used for further constructs. ~~Various~~ SEQ ID NO:2 and SEQ ID NO:3, two ~~and deletion~~ fragments of ~~pGPlas3-2~~ SEQ ID NO:1 were operably ~~transcriptionally and transitionally~~ fused at the 5'terminus of the GUS reporter gene in pBI201 by RCR ligation, and ~~these~~ resulting constructs were used for transitory expression studies using DNA bombardment. ~~Upon identification of the adequate deletion fragment, it was~~ or subcloned into a binary plant expression vector such as pBI101 (Clonetech). These recombinant plasmids were used for stable integration through *A. tumefaciens* infection as described below.

Agrobacterium-mediated DNA transfer and regeneration of transgenic lines

The recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain LBA4404 by electroporation as described in Khoudi et al (1999, *Biotechnol. Bioeng.*, 64:135-143). Selected *Agrobacterium* strains were then co-cultivated with leaf disks from genotype C5-1 for 4 days in the absence of selection pressure (kanamycin). Following this incubation period, leaf disks were washed and pampered, and then allowed to form calli onto medium B5H. Calli were then transferred for 21 days on SH medium for embryo induction and for 28 days on BOi2Y for embryo development. Torpedo-shaped embryos were removed from Boi2Y and placed on MS medium for regeneration. Kanamycin was present in all cultivation medium except for co-cultivation and regeneration on MS. This method is described in length in Desgagnés et al (1995, *Plant Cell Tissue Organ Cult.* 42:129-140). Rooted plantlets were grown to maturity in the greenhouse.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.